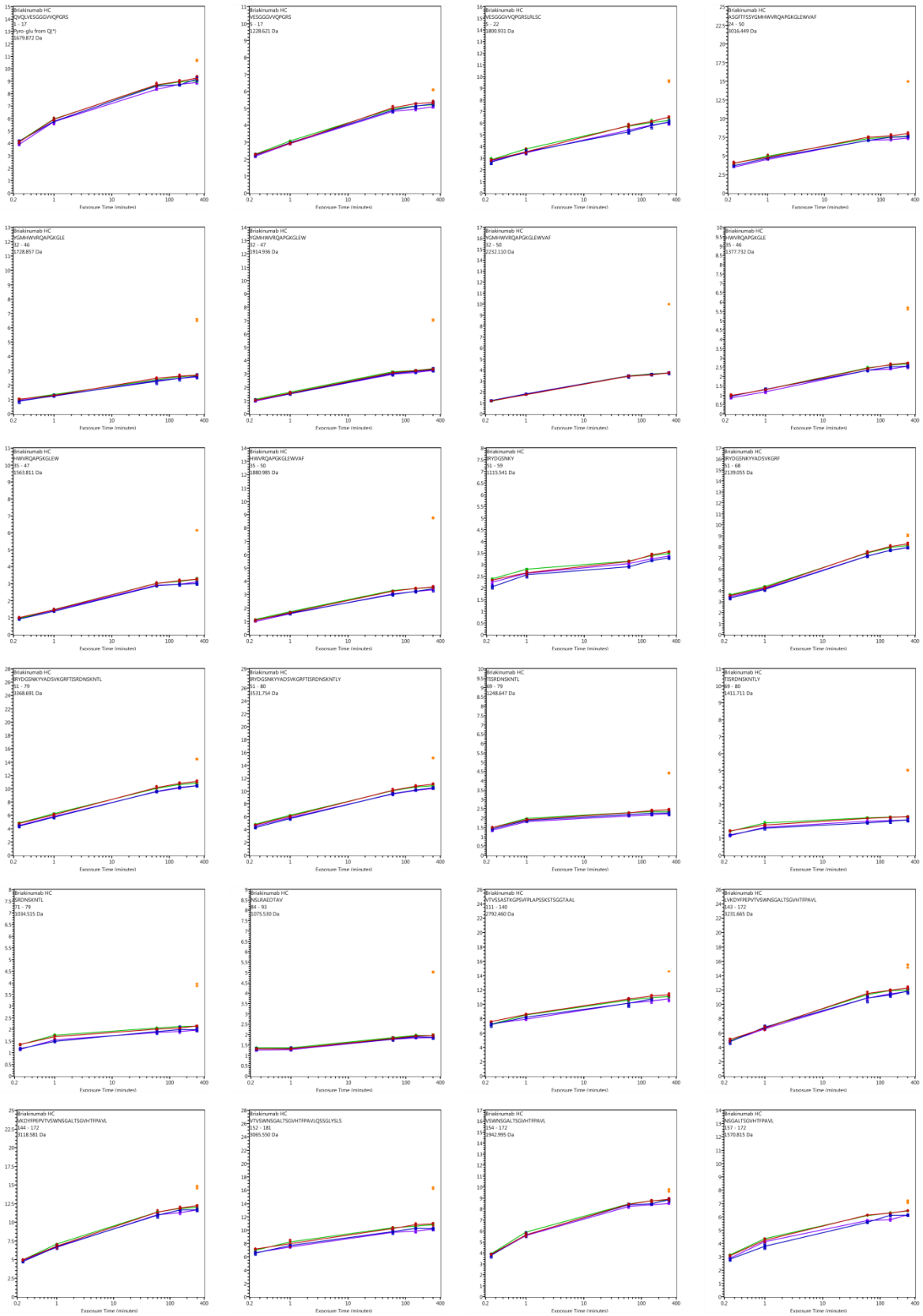
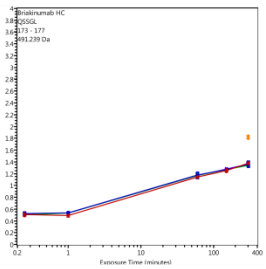
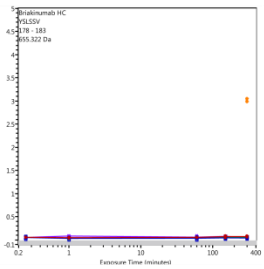
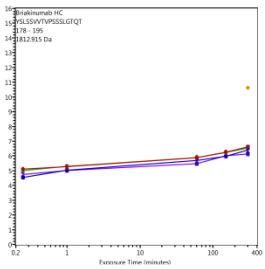
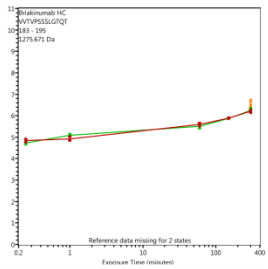
Supplemental information

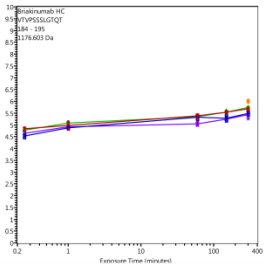

Briakinumab HC
QVQLVESGGGVVQPGRS
1 - 17
Pyro-glu from Q(*)
1679.872 Da
Briakinumab HC
VESGGGVVQPGRS
5 - 17
1228.621 Da
Briakinumab HC
VESGGGVVQPGRSLRLSC
5 - 22
1800.931 Da
Briakinumab HC
ASGITFSSYGMHWVRQAPGKGLEWVAF
24 - 50
2816.449 Da
Briakinumab HC
YGMHWVRQAPGKGLE
32 - 46
1728.857 Da
Briakinumab HC
YGMHWVRQAPGKGLEW
32 - 47
1914.936 Da
Briakinumab HC
YGMHWVRQAPGKGLEWVAF
32 - 50
2332.110 Da
Briakinumab HC
HWVRQAPGKGLE
35 - 46
1377.732 Da
Briakinumab HC
HWVRQAPGKGLEW
35 - 47
1563.811 Da
Briakinumab HC
HWVRQAPGKGLEWVAF
35 - 50
1880.985 Da
Briakinumab HC
RYDGSNKY
51 - 59
1115.541 Da
Briakinumab HC
RYDGSNKYYADSVKGRF
51 - 68
2139.055 Da
Briakinumab HC
RYDGSNKYYADSVKGRFTISRDNSKNTL
51 - 79
2968.691 Da
Briakinumab HC
RYDGSNKYYADSVKGRFTISRDNSKNTLY
51 - 80
3531.754 Da
Briakinumab HC
TISRDNSKNTL
69 - 79
1248.647 Da
Briakinumab HC
TISRDNSKNTLY
69 - 80
1411.711 Da
Briakinumab HC
SRDNSKNTL
71 - 79
1034.515 Da
Briakinumab HC
NSLRAEDTAV
84 - 93
1075.530 Da
Briakinumab HC
VTVSSASTKGPSVFPLAPSSKSTSGGTAAL
111 - 140
2792.460 Da
Briakinumab HC
VKDYYPEPVTVSWNSGALTSGVHTFPAVL
143 - 172
3231.665 Da
Briakinumab HC
VKDYYPEPVTVSWNSGALTSGVHTFPAVL
144 - 172
3118.581 Da
Briakinumab HC
VTVSWNSGALTSGVHTFPAVLQSSGLYSLS
152 - 181
3065.550 Da
Briakinumab HC
VSWNSGALTSGVHTFPAVL
154 - 172
1942.995 Da
Briakinumab HC
NSGALTSGVHTFPAVL
157 - 172
1570.815 Da
Exposure Time (minutes)

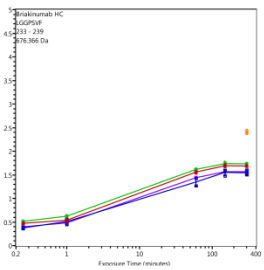

Briakinumab HC
QSSGL
173 - 177
581.239 Da
Exposure Time (minutes)

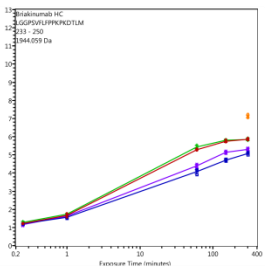

Briakinumab HC
YSLSSV
178 - 183
655.322 Da
Exposure Time (minutes)

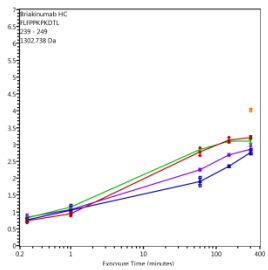

Briakinumab HC
VSLSSVVTVPSSSLGTQT
178 - 195
1812.915 Da
Exposure Time (minutes)

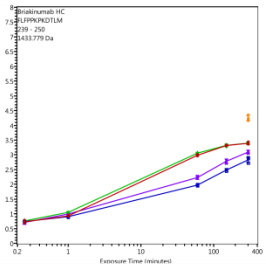

Briakinumab HC
VVTVPSSSLGTQT
183 - 195
1275.671 Da
Reference data missing for 2 states
Exposure Time (minutes)

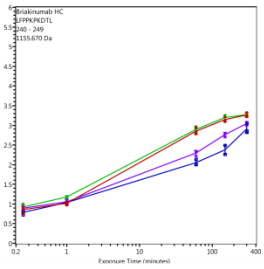

Briakinumab HC
VTVPSSSLGTQT
184 - 195
1176.603 Da
Exposure Time (minutes)

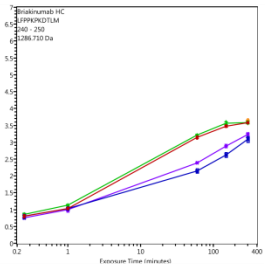

Briakinumab HC
LGGPSVF
233 - 239
676.366 Da
Exposure Time (minutes)

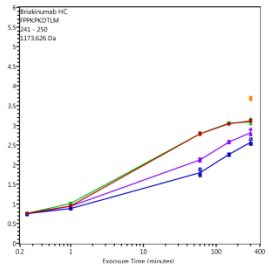

Briakinumab HC
LGGPSVFLFPPKPKDTLM
233 - 250
1944.059 Da
Exposure Time (minutes)

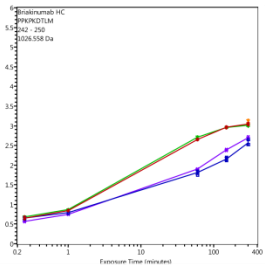

Briakinumab HC
LFPPKPKDTL
239 - 249
1302.738 Da
Exposure Time (minutes)

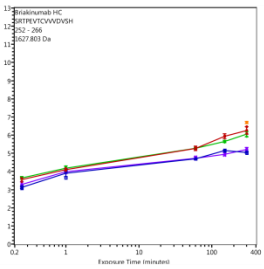

Briakinumab HC
LFPPKPKDTLM
239 - 250
1433.779 Da
Exposure Time (minutes)

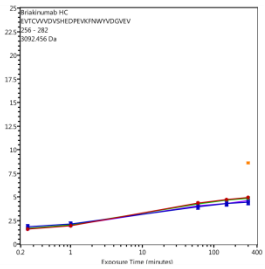

Briakinumab HC
FPPKPKDTL
240 - 249
1155.670 Da
Exposure Time (minutes)

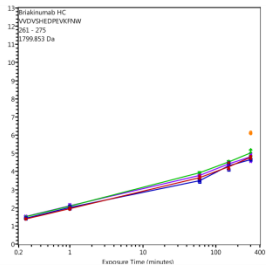

Briakinumab HC
FPPKPKDTLM
240 - 250
1286.710 Da
Exposure Time (minutes)

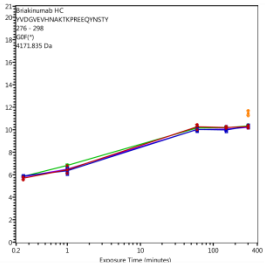

Briakinumab HC
PPKPKDTLM
241 - 250
1173.626 Da
Exposure Time (minutes)

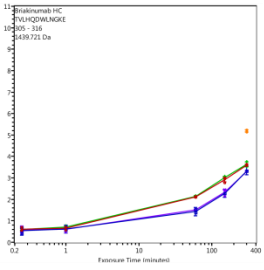

Briakinumab HC
PPKPKDTLM
242 - 250
1026.558 Da
Exposure Time (minutes)

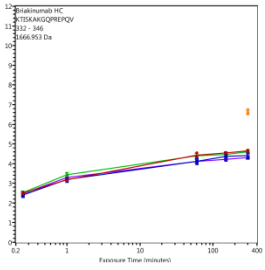

Briakinumab HC
RTPEVTCVVVDVSH
252 - 266
1627.803 Da
Exposure Time (minutes)

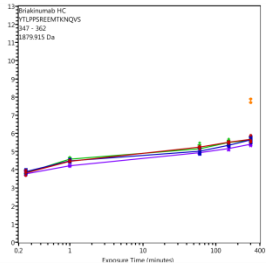

Briakinumab HC
VTCVVVDVSHEDPEVKFNWYVDGVEV
256 - 282
3092.456 Da
Exposure Time (minutes)

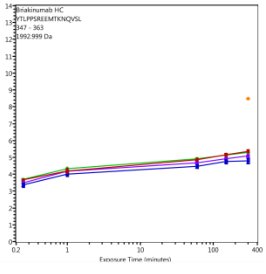

Briakinumab HC
VDVSHEDPEVKFNW
261 - 275
1799.853 Da
Exposure Time (minutes)

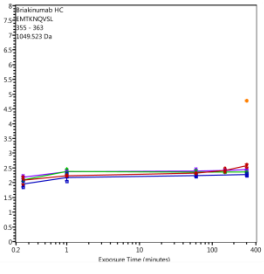

Briakinumab HC
VDGVEVHNAKTKPREEQYNSTY
276 - 298
(OPI*)
4171.835 Da
Exposure Time (minutes)

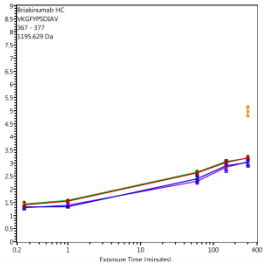

Briakinumab HC
VLHQDWLNGKE
305 - 316
1439.721 Da
Exposure Time (minutes)

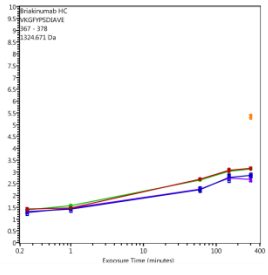

Briakinumab HC
KTISKAKGQPREPQV
332 - 346
1666.953 Da
Exposure Time (minutes)


Briakinumab HC
TLPPSREEMTKNQVS
347 - 362
1879.915 Da
Exposure Time (minutes)


Briakinumab HC
TLPPSREEMTKNQVSL
347 - 363
1992.999 Da
Exposure Time (minutes)


Briakinumab HC
EMTKNQVSL
355 - 363
1049.523 Da
Exposure Time (minutes)


Briakinumab HC
VKGFYPSDIAV
367 - 377
1195.629 Da
Exposure Time (minutes)


Briakinumab HC
VKGFYPSDIAVE
367 - 378
1324.671 Da
Exposure Time (minutes)

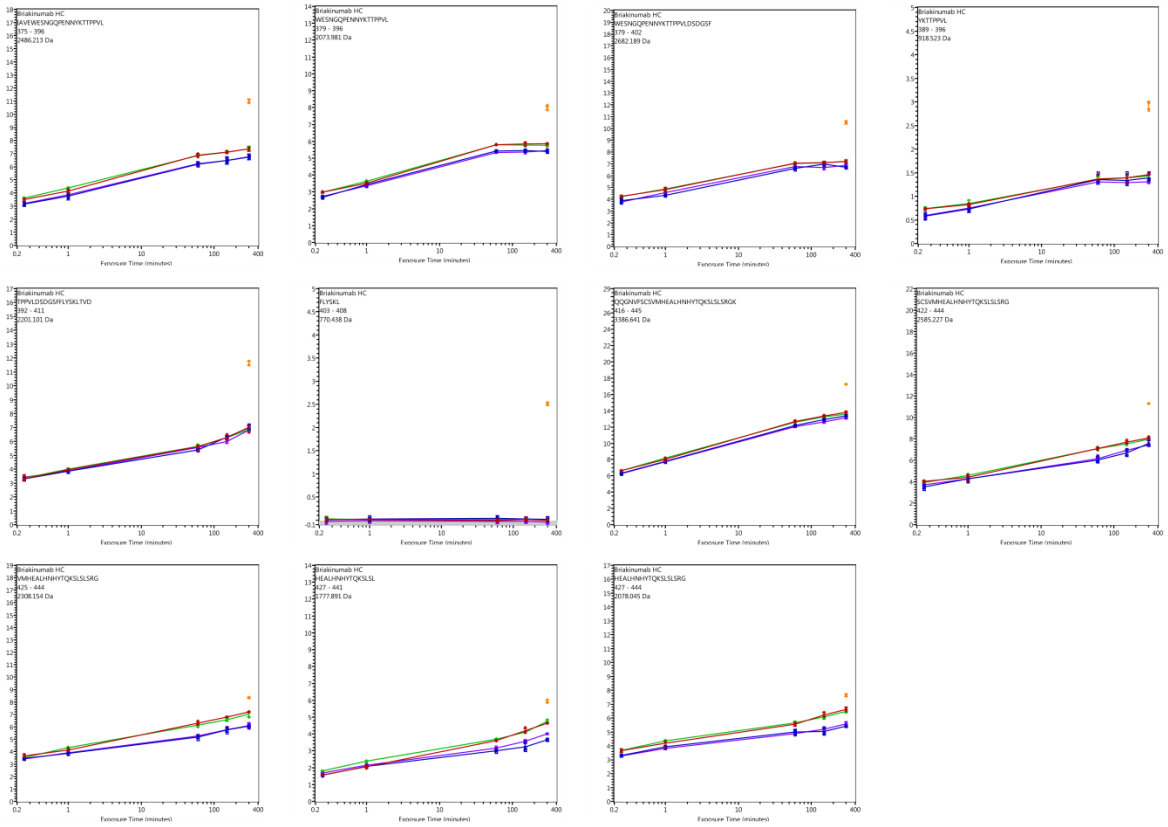
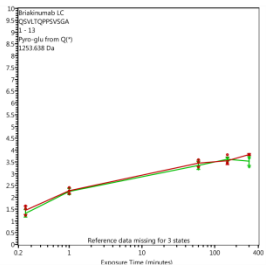
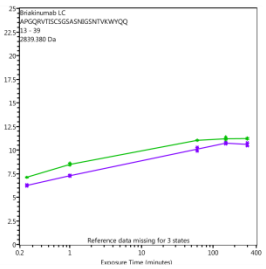
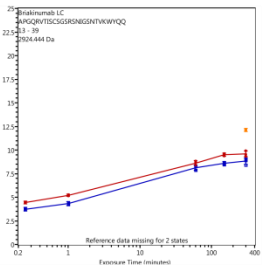
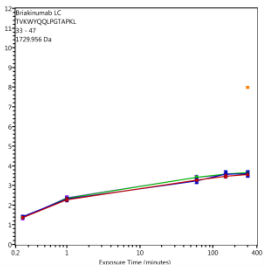


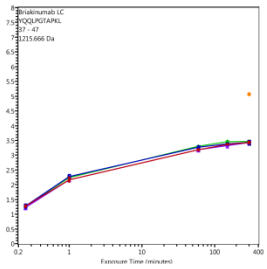
Figure S1. Deuterium uptake plots of heavy chain briakinumab and the briakinumab variant peptides. Deuterium incorporation of unbound briakinumab (red), FcRn-bound briakinumab (blue), unbound briakinumab variant (green), FcRn-bound briakinumab variant (purple) was determined in triplicate experiments at 15 s, 1 min, 1 h, 2.5 h and 5h and plotted with 1x standard deviation (in most cases the SD was lower than the size of the data point marker). The 90 % D_2O control is shown in orange at the last time point.

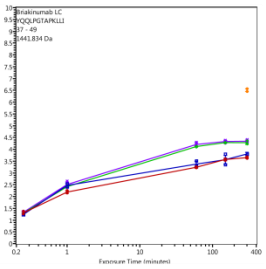

Briakinumab LC
QSVLTQPPSVSGA
1 - 13
Pyro-glu from Q(*)
1253.638 Da
Reference data missing for 3 states
Exposure Time (minutes)

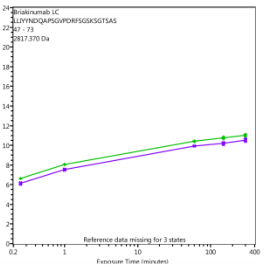

Briakinumab LC
APGQRVTISCSGSASNIGSNTVKWYQQ
13 - 39
2839.380 Da
Reference data missing for 3 states
Exposure Time (minutes)

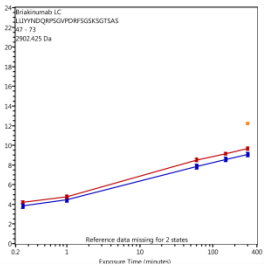

Briakinumab LC
APGQRVTISCSGSRSNIGSNTVKWYQQ
13 - 39
2924.444 Da
Reference data missing for 2 states
Exposure Time (minutes)

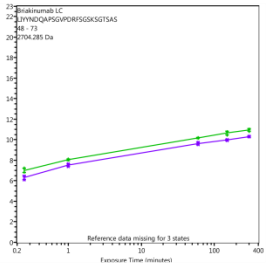

Briakinumab LC
TVKWYQQLPGTAPKL
33 - 47
1729.956 Da
Exposure Time (minutes)

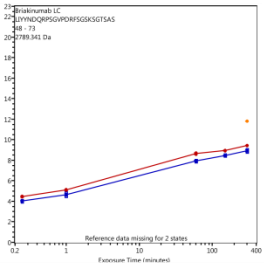

Briakinumab LC
YQQLPGTAPKL
37 - 47
1215.666 Da
Exposure Time (minutes)

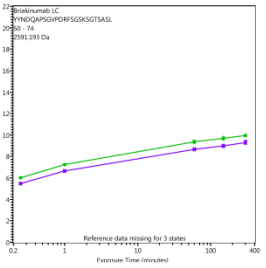

Briakinumab LC
YQQLPGTAPKLLI
37 - 49
1441.834 Da
Exposure Time (minutes)

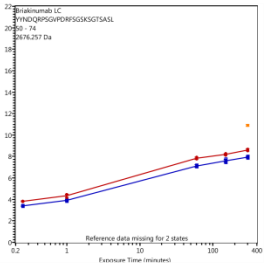

Briakinumab LC
LIYYNDQAPSGVPDRFSGSKSGTSAS
47 - 73
2817.370 Da
Reference data missing for 3 states
Exposure Time (minutes)

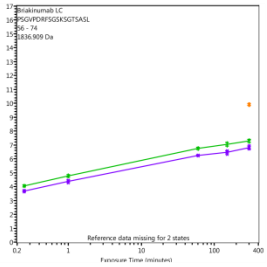

Briakinumab LC
LIYYNDQDPSGVPDRFSGSKSGTSAS
47 - 73
2902.425 Da
Reference data missing for 2 states
Exposure Time (minutes)

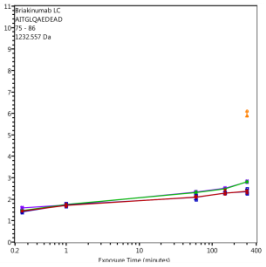

Briakinumab LC
LIYYNDQAPSGVPDRFSGSKSGTSAS
48 - 73
2704.285 Da
Reference data missing for 3 states
Exposure Time (minutes)

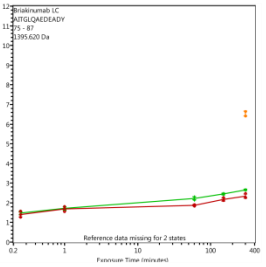

Briakinumab LC
LIYYNDQRPSGVPDRFSGSKSGTSAS
48 - 73
2789.341 Da
Reference data missing for 2 states
Exposure Time (minutes)

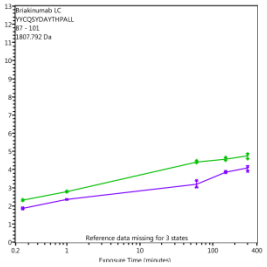

Briakinumab LC
YYNDQAPSGVPDRFSGSKSGTSASL
50 - 74
2991.193 Da
Reference data missing for 3 states
Exposure Time (minutes)

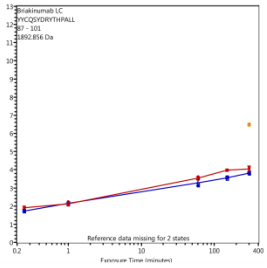

Briakinumab LC
YYNDQRPSGVPDRFSGSKSGTSASL
50 - 74
2876.257 Da
Reference data missing for 2 states
Exposure Time (minutes)

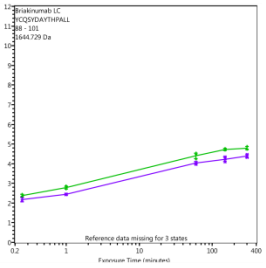

Briakinumab LC
PSGVPDRFSGSKSGTSASL
56 - 74
1836.909 Da
Reference data missing for 2 states
Exposure Time (minutes)

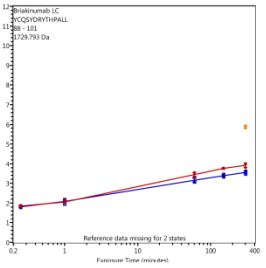

Briakinumab LC
ATISGLQAEDEAD
75 - 86
1232.557 Da
Exposure Time (minutes)

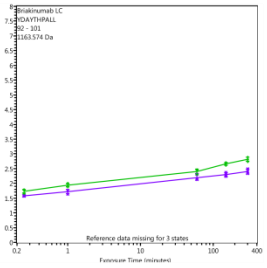

Briakinumab LC
ATISGLQAEDEADY
75 - 87
1395.620 Da
Reference data missing for 2 states
Exposure Time (minutes)

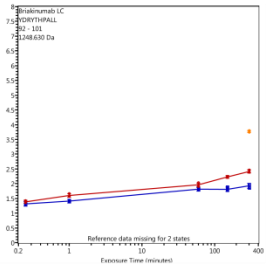

Briakinumab LC
YYCQSYDAYTHPALL
87 - 101
1807.792 Da
Reference data missing for 3 states
Exposure Time (minutes)

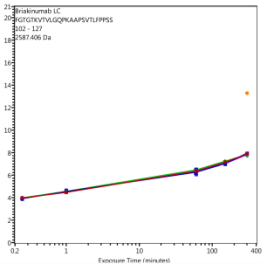

Briakinumab LC
YYCQSYDRYTHPALL
87 - 101
1892.856 Da
Reference data missing for 2 states
Exposure Time (minutes)

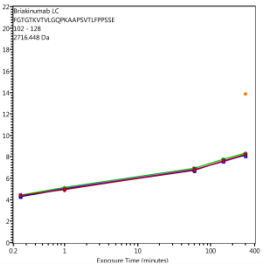

Briakinumab LC
YCQSYDAYTHPALL
88 - 101
1644.729 Da
Reference data missing for 3 states
Exposure Time (minutes)

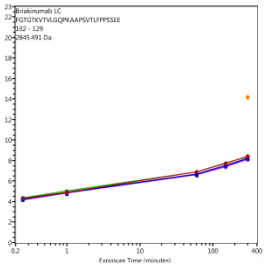

Briakinumab LC
YCQSYDRYTHPALL
88 - 101
1729.793 Da
Reference data missing for 2 states
Exposure Time (minutes)


Briakinumab LC
DAYTHPALL
92 - 101
1163.574 Da
Reference data missing for 3 states
Exposure Time (minutes)


Briakinumab LC
YDRYTHPALL
92 - 101
1248.630 Da
Reference data missing for 2 states
Exposure Time (minutes)


Briakinumab LC
GTGTKVTVLGQPKAAPSVTLFPPSS
102 - 127
2587.406 Da
Exposure Time (minutes)


Briakinumab LC
TGTGTKVTVLGQPKAAPSVTLFPPSSE
102 - 128
2716.448 Da
Exposure Time (minutes)


Briakinumab LC
GTGTKVTVLGQPKAAPSVTLFPPSSEE
102 - 129
2845.491 Da
Exposure Time (minutes)

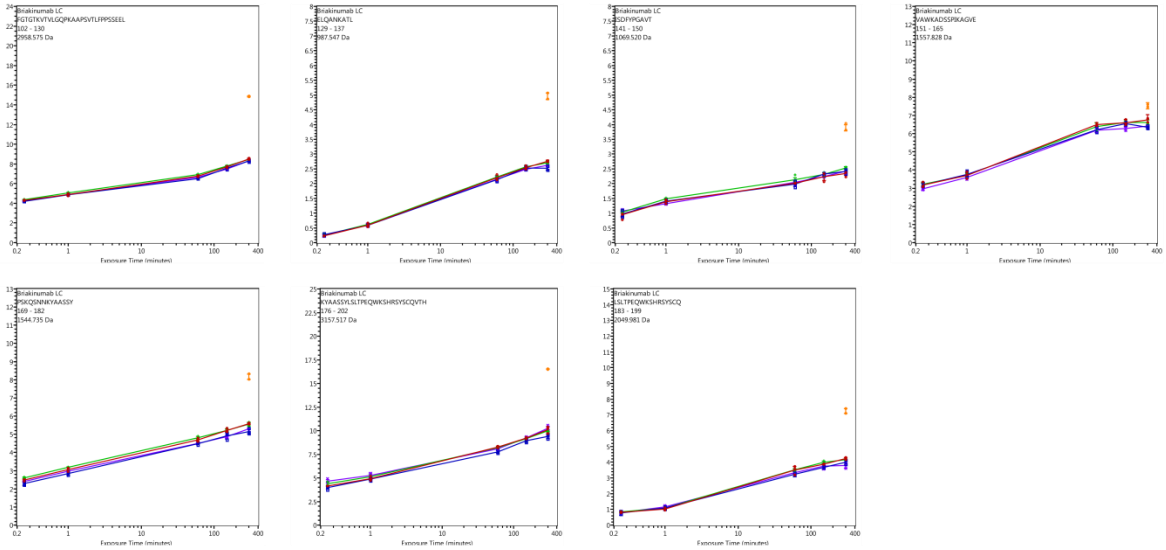
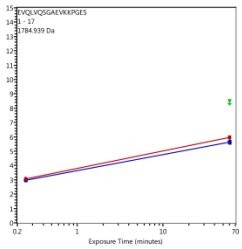
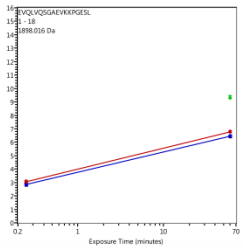
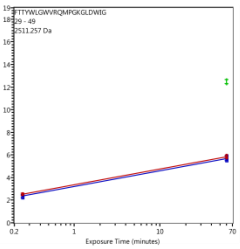


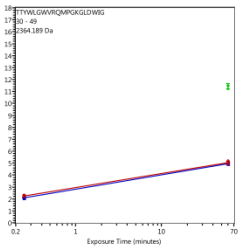
Figure S2. Deuterium uptake plots of light chain briakinumab and the briakinumab variant peptides. Deuterium incorporation of unbound briakinumab (red), FcRn-bound briakinumab (blue), unbound briakinumab variant (green), FcRn-bound briakinumab variant (purple) was determined in triplicate experiments at 15 s, 1 min, 1 h, 2.5 h and 5h and plotted with 1x standard deviation (in most cases the SD was lower than the size of the data point marker). The 90 % D_2O control is shown in orange at the last time point.

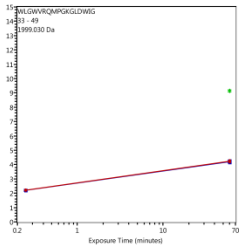
Figure S3. Sequence coverage map of briakinumab and the briakinumab variant A) heavy chain and B) light chain.

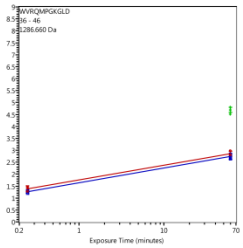

EVQLVQSGAEVKKPGES
1 - 17
1784.939 Da
Exposure Time (minutes)

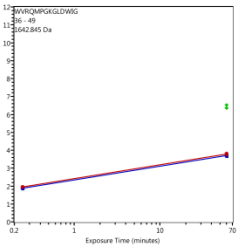

EVQLVQSGAEVKKPGESL
1 - 18
1898.016 Da
Exposure Time (minutes)

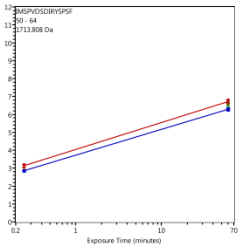

FTFYWLGWVRQMPGKGLDWIG
29 - 49
2511.257 Da
Exposure Time (minutes)

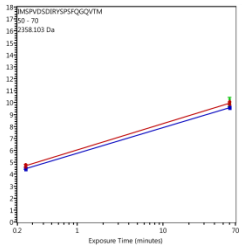

FTFYWLGWVRQMPGKGLDWIG
30 - 49
2364.189 Da
Exposure Time (minutes)

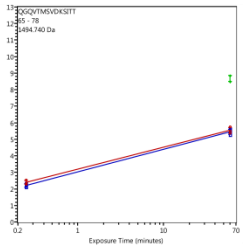

WLGWVRQMPGKGLDWIG
33 - 49
1999.030 Da
Exposure Time (minutes)

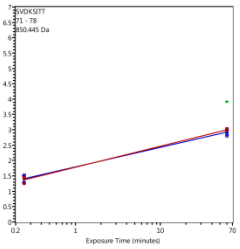

WVRQMPGKGLD
36 - 46
1286.660 Da
Exposure Time (minutes)

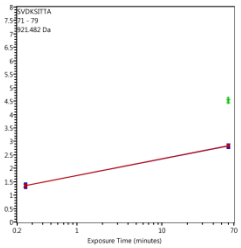

WVRQMPGKGLDWIG
36 - 49
1642.845 Da
Exposure Time (minutes)

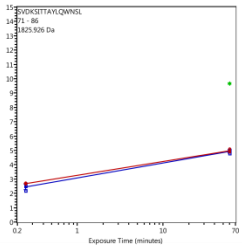

MSPVDSDIRYSPSF
50 - 64
1713.808 Da
Exposure Time (minutes)

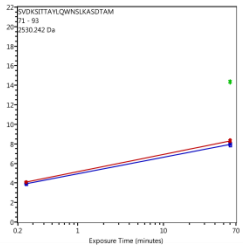

MSPVDSDIRYSPSFQSGQVTM
50 - 70
2358.103 Da
Exposure Time (minutes)

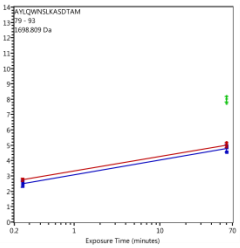

SKGQVTMSVDKSITT
65 - 78
1494.740 Da
Exposure Time (minutes)

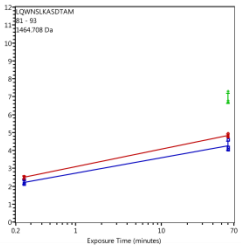

SVDKSITT
71 - 78
950.445 Da
Exposure Time (minutes)

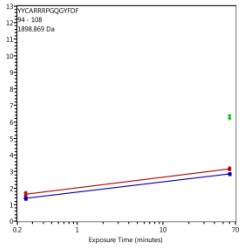

SVDKSITTA
71 - 79
1021.482 Da
Exposure Time (minutes)

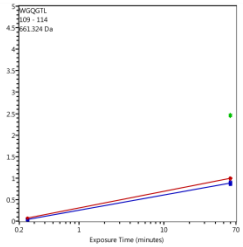

SVDKSITTAYLQWNSL
71 - 86
1825.926 Da
Exposure Time (minutes)

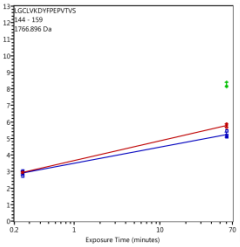

VDKSITTAYLQWNSLKASDTAM
72 - 93
2530.242 Da
Exposure Time (minutes)

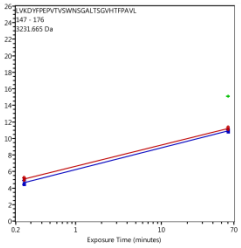

AYLQWNSLKASDTAM
79 - 93
1698.809 Da
Exposure Time (minutes)

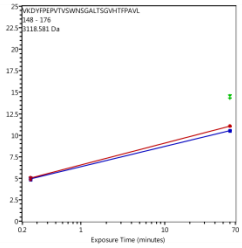

LQWNSLKASDTAM
81 - 93
1464.708 Da
Exposure Time (minutes)

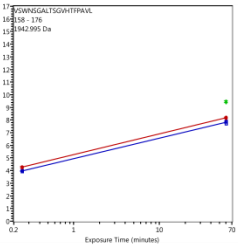

YFCARRRPGQGVTDF
94 - 108
1898.869 Da
Exposure Time (minutes)

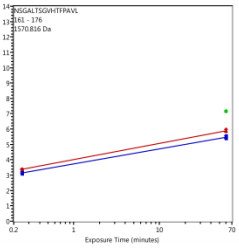

WGQGTL
109 - 114
661.324 Da
Exposure Time (minutes)

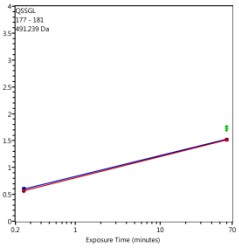

LGCLVKDYFPEPVTVS
144 - 159
1766.896 Da
Exposure Time (minutes)

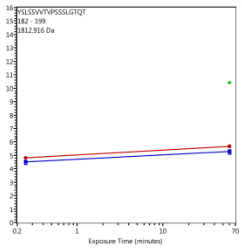

LVKDYFPEPVTVSWNSGALTSGVHTFPAVL
147 - 176
3231.665 Da
Exposure Time (minutes)

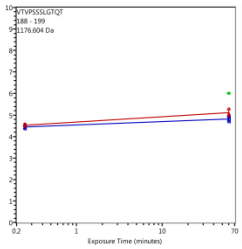

VKDYFPEPVTVSWNSGALTSGVHTFPAVL
148 - 176
3118.581 Da
Exposure Time (minutes)

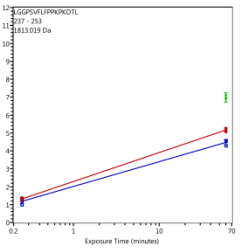

VSWNSGALTSGVHTFPAVL
158 - 176
1942.995 Da
Exposure Time (minutes)

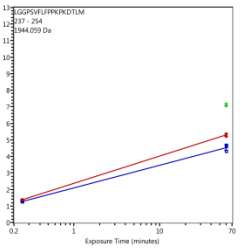

NSGALTSGVHTFPAVL
161 - 176
1570.816 Da
Exposure Time (minutes)

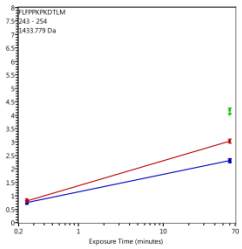

QSSGL
177 - 181
491.239 Da
Exposure Time (minutes)

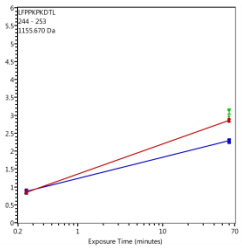

VSLSSVVTVPSSSLGTQT
182 - 199
1812.916 Da
Exposure Time (minutes)

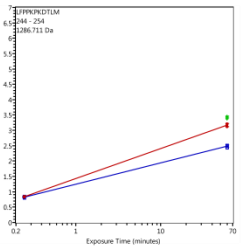

VTVPSSSLGTQT
188 - 199
1176.604 Da
Exposure Time (minutes)

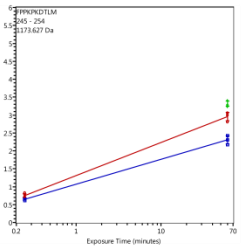

LGGPSVFLFPPKPKDTL
237 - 253
1813.019 Da
Exposure Time (minutes)

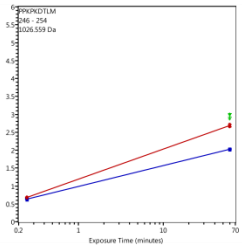

LGGPSVFLFPPKPKDTLM
237 - 254
1944.059 Da
Exposure Time (minutes)

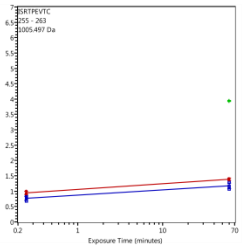

FLFPPKPKDTLM
243 - 254
1433.779 Da
Exposure Time (minutes)

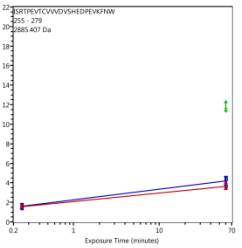

FPPKPKDTL
244 - 253
1155.670 Da
Exposure Time (minutes)

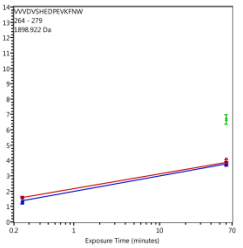

FPPKPKDTLM
244 - 254
1286.711 Da
Exposure Time (minutes)

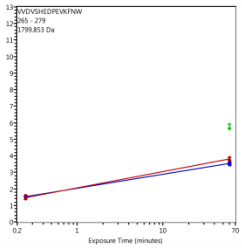

PPKPKDTLM
245 - 254
1173.627 Da
Exposure Time (minutes)

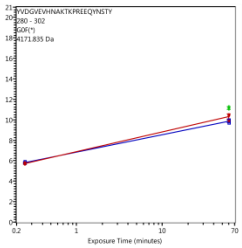

PKPKDTLM
246 - 254
1026.559 Da
Exposure Time (minutes)

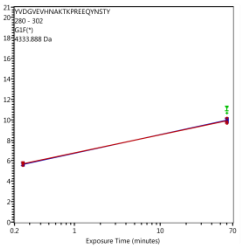

RTPEVTC
255 - 263
1005.497 Da
Exposure Time (minutes)

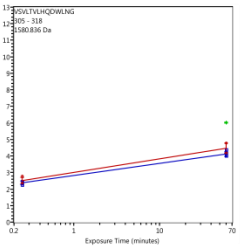

RTPEVTCVVVDVSHEDPEVKFNW
255 - 279
2885.407 Da
Exposure Time (minutes)

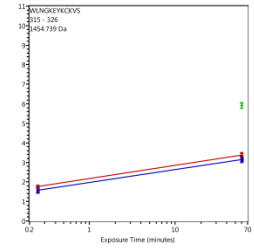

VVVDVSHEDPEVKFNW
264 - 279
1898.922 Da
Exposure Time (minutes)

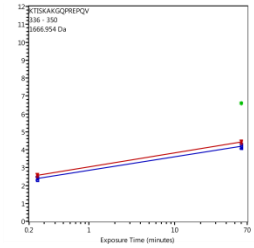

VDVSHEDPEVKFNW
265 - 279
1799.853 Da
Exposure Time (minutes)

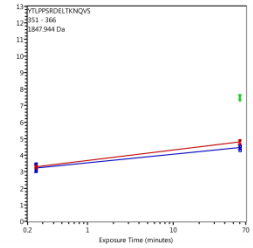

VDGVEVHNAKTKPREEQYNSTY
280 - 302
1GOF(*)
2417.835 Da
Exposure Time (minutes)

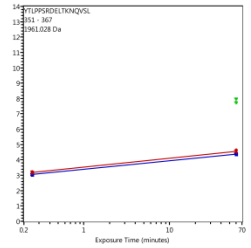

VDGVEVHNAKTKPREEQYNSTY
280 - 302
1GIF(*)
2333.888 Da
Exposure Time (minutes)

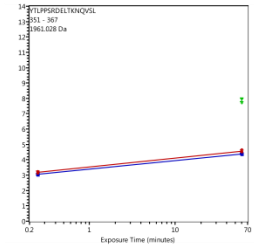

VDVLTVLHQDWLNG
305 - 318
1580.836 Da
Exposure Time (minutes)

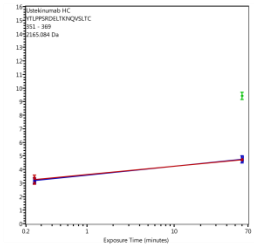

WLNGKEYKCKVS
315 - 326
1454.739 Da
Exposure Time (minutes)

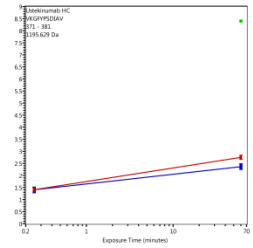

KTISKAKGQPREPQV
336 - 350
1668.954 Da
Exposure Time (minutes)

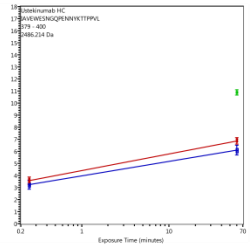

YTLPPSRDELTKNQVS
351 - 366
1847.944 Da
Exposure Time (minutes)


YTLPPSRDELTKNQVSL
351 - 367
1961.028 Da
Exposure Time (minutes)


YTLPPSRDELTKNQVSL
351 - 367
1961.028 Da
Exposure Time (minutes)


Astekinumab HC
YTLPPSRDELTKNQVSLTC
351 - 369
2165.084 Da
Exposure Time (minutes)


Astekinumab HC
VEGFYPSDIAV
371 - 381
1195.629 Da
Exposure Time (minutes)


Astekinumab HC
AVEWESNGQPENNYKTTPPVL
379 - 400
2486.214 Da
Exposure Time (minutes)

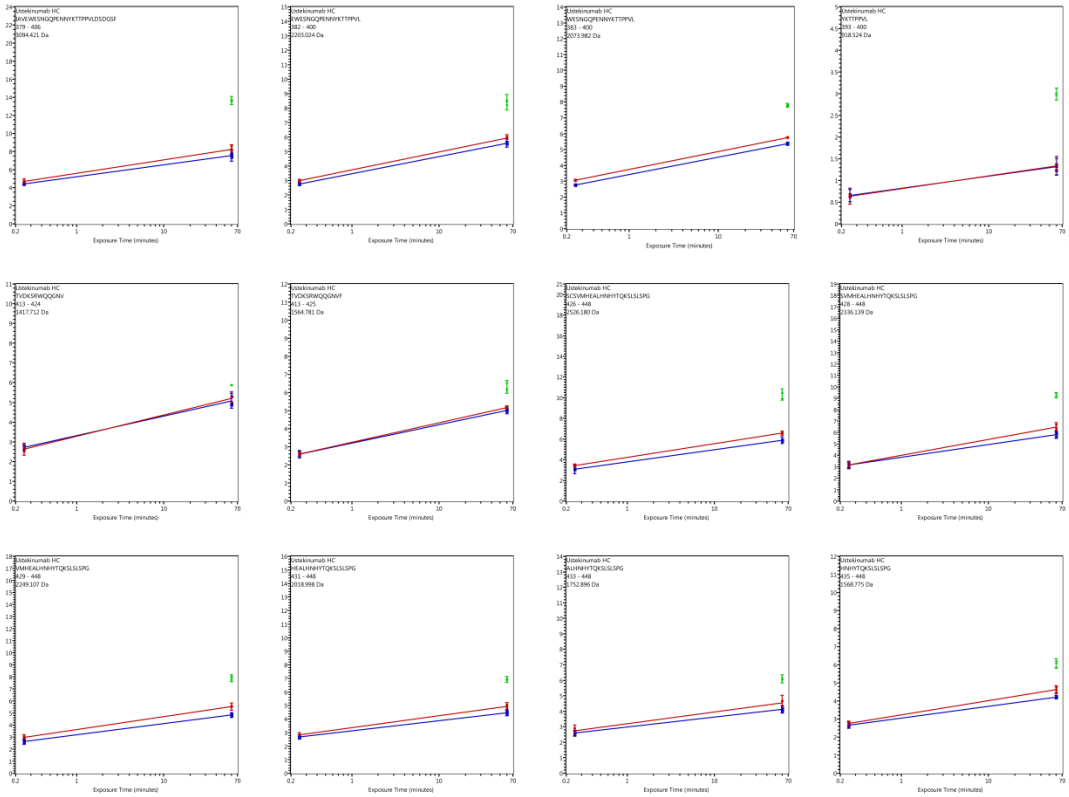


Figure S4. Deuterium uptake plots of heavy chain ustekinumab peptides. Deuterium incorporation of unbound ustekinumab (red), FcRn-bound ustekinumab (blue) was determined in triplicate experiments at 15 s and 1 h and plotted with 1x standard deviation (in most cases the SD was lower than the size of the data point marker). The 90 % D_2O control is shown in green at the last time point.

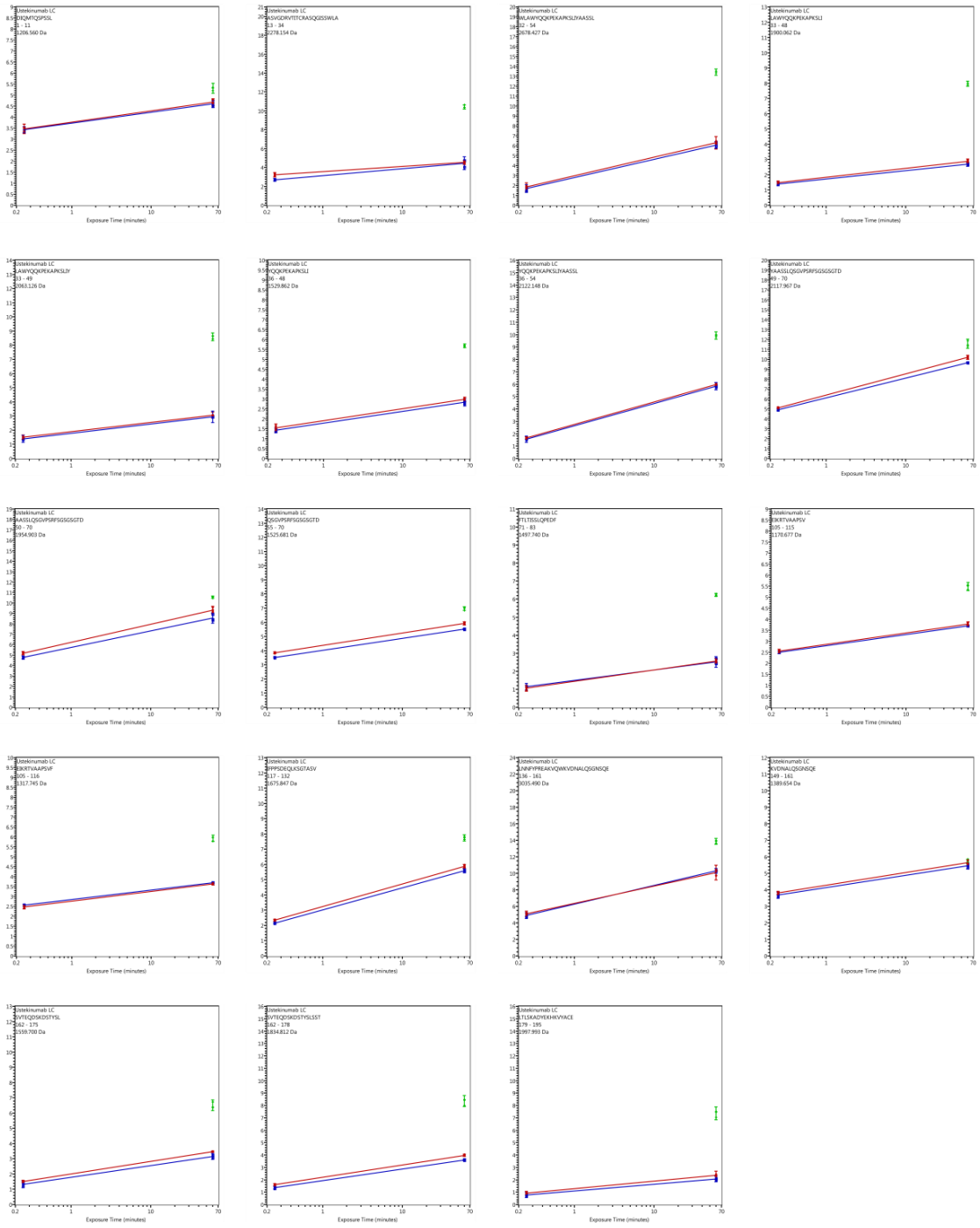


Figure S5. Deuterium uptake plots of light chain ustekinumab peptides. Deuterium incorporation of unbound ustekinumab (red), FcRn-bound ustekinumab (blue) was determined in triplicate experiments at 15 s and 1 h. and

plotted with 1x standard deviation (in most cases the SD was lower than the size of the data point marker). The 90 % D_2O control is shown in green at the last time point.

Figure S6. Sequence coverage map of ustekinumab A) heavy chain and B) light chain.

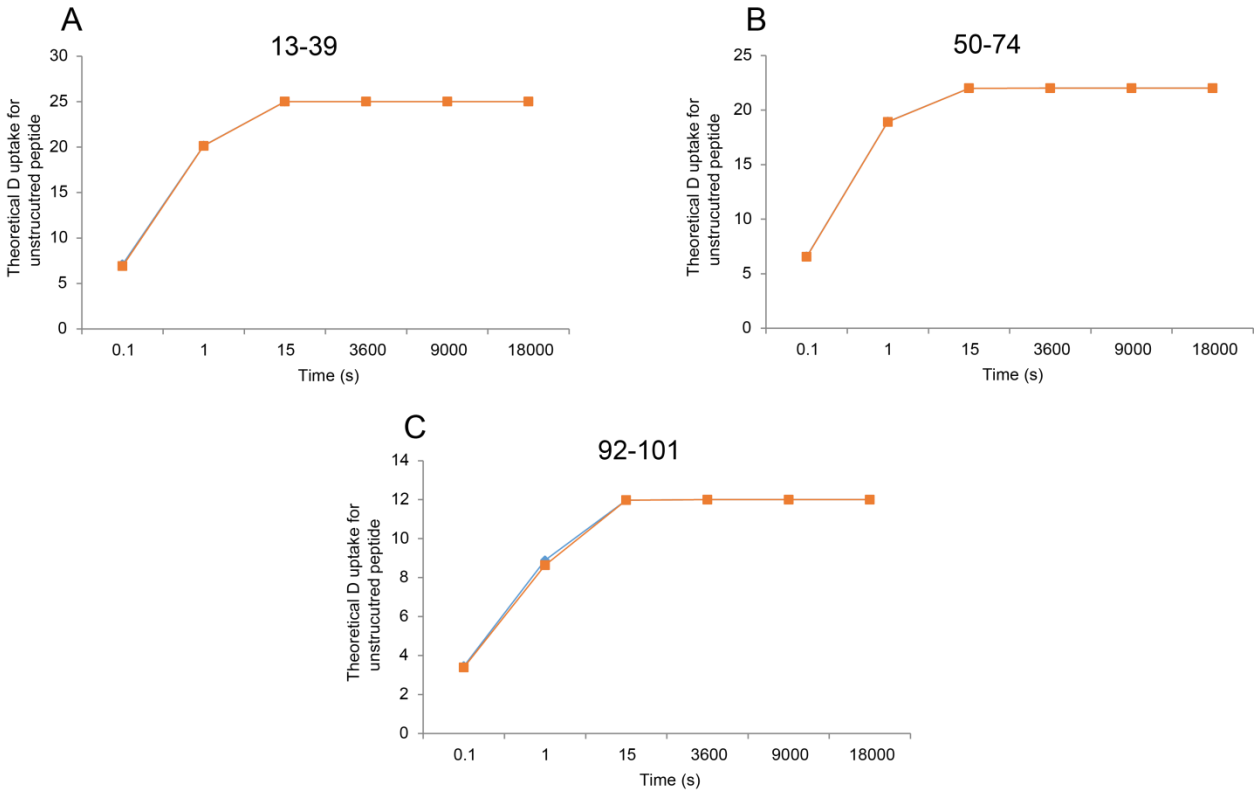


Figure S7. Comparison of theoretical deuterium uptake of unstructured peptides of briakinumab and the briakinumab variant. Theoretical deuterium uptake of peptides containing the R to A substitution: A) 13-39, B) 50-74 and C) 92-101 of briakinumab (blue) and the briakinumab variant (orange) at time points 0.1 s, 1 s, 15 s, 1 h, 2.5 h and 5 h. The theoretical uptake for random coil peptides was calculated using intrinsic exchange rates of Bai et al. (18)

```
Bria    -QSVLTQPPSVSGAPGQRVTISCSGSRSNIGSNTVKWYQQLPGTAPKLLIYYNDQRPSGV   59
Uste    DIQMTQSPSSLSASVGDRVTITCRASQG--ISSWLAWYQQKPEKAPKSLIYAASSLQSGV   58
          .:  .* *:*.: *:****:* .*:.   *. : **** * .*** ***  ..  ***

Bria    PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDRYTHPALLFGTGTKVTVLGQPKAAPS   119
Uste    PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNIYP---YTFGQGTKLEI-KRTVAAPS   114
        *.***** ***. :*:*:.** ** * ****.*: *      ** ***: :  :  ****

Bria    VTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPIKAGVE-TTTPSKQSNNKYA   178
Uste    VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS   174
        * :****.*:*:: .*::***:.:***  ..* **.*.:  ..  : :.* ....:..*:

Bria    ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP-----   213
Uste    LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC   214
         ** *:*:  :::.*: *:*:***:* :   * :
```

Figure S8. Sequence alignment of ustekinumab and briakinumab LC. Sequence alignment performed with Clustal Omega. (*) indicates a fully conserved residue, (:) indicates conservation between residues with strongly similar properties, and (.) indicates conservation between residues with weakly similar properties. (20)